

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

DTIC FILE 000

(2)

DOCUMENTATION PAGE

1a. REPORT AD-A200 910		1b. RESTRICTIVE MARKINGS	
2a. SECUR		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.	
2b. DECL		5. MONITORING ORGANIZATION REPORT NUMBER(S) ARO 24463.2-LS	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) DG		7a. NAME OF MONITORING ORGANIZATION U. S. Army Research Office	
6a. NAME OF PERFORMING ORGANIZATION Columbia University College of Physicians & Surgeons		7b. ADDRESS (City, State, and ZIP Code) P. O. Box 12211 Research Triangle Park, NC 27709-2211	
6b. ADDRESS (City, State, and ZIP Code) 630 West 168th Street New York, NY 10032		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAAL03-86-K-0162	
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U. S. Army Research Office		10. SOURCE OF FUNDING NUMBERS	
8b. ADDRESS (City, State, and ZIP Code) P. O. Box 12211 Research Triangle Park, NC 27709-2211		PROGRAM ELEMENT NO	PROJECT NO
		TASK NO	WORK UNIT ACCESSION NO
11. TITLE (Include Security Classification) Surface Processes in Membrane Transport			
12. PERSONAL AUTHOR(S) Martin Blank			
13a. TYPE OF REPORT Final	13b. TIME COVERED FROM 9/1/86 TO 8/31/88	14. DATE OF REPORT (Year, Month, Day) 1988 Oct. 31	15. PAGE COUNT 3
16. SUPPLEMENTARY NOTATION The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) This report summarizes the results of theoretical and experimental investigations of the role of charged surfaces on membrane processes. The focus on electrical double layer processes of the channels involved in ion transport and excitation leads to a relation between the ion selectivity and the rate of channel opening. Recent studies show that similar electrochemical processes may be occurring in biosynthetic structures and causing the changes seen in the proteins of cells exposed to electromagnetic signals. <i>Keywords: Ion channels, Electrophysiology, Stimulation Physiology.</i> (AW)			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL		22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL

SURFACE PROCESSES IN MEMBRANE TRANSPORT

FINAL REPORT

October 31, 1988

U. S. ARMY RESEARCH OFFICE

CONTRACT NUMBER DAAL03-86-K-0162

COLUMBIA UNIVERSITY

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DESIGNATED BY OTHER DOCUMENTATION.

1. STATEMENT OF THE PROBLEM STUDIED

The effect of molecular charge on protein aggregation reactions provides an approach to understanding the opening/closing reactions of voltage gated channels. The surface charge also affects the electrochemical gradients across membranes during transients and the ionic fluxes. Using theoretical and experimental approaches, we have studied the role of charged surfaces in membrane processes.

2. SUMMARY OF THE MOST IMPORTANT RESULTS

2.1 - The relation between surface charge density and protein aggregation has been verified experimentally for the hemoglobin tetramer \rightleftharpoons dimer reaction. This provides a basis for understanding the energetics of membrane channel opening/closing reactions, and their relation to charging.

2.2 - The ion selectivity of voltage gated channels appears to be related to the gating current. Since the electrochemical gradients across the channels vary with the time after electrical stimulation, the effective gradient depends upon the gating current, which sets the time when the channels open.

2.3 - The same processes that lead to the opening/closing of channels in membranes apparently occur in biosynthetic structures under the influence of imposed electromagnetic fields and lead to changes in the patterns of transcription and translation.

3. DESCRIPTION OF RESEARCH

3.1 - MEMBRANE CHANNEL PROCESSES

The fundamental membrane processes of living cells, e.g. generation of ion gradients, sensory transduction, conduction of impulses, energy transduction, are electrochemical in nature and involve ion movement through specialized protein assemblies called channels. There are structural similarities between channels that suggest a common design and mode of operation. We have studied protein aggregation/disaggregation reactions as a basis for channel function and to establish an electrochemical link between electrical stimuli and channel properties.

We have measured osmotically the disaggregation of hemoglobin tetramers into dimers as a function of pH, and have calculated the equilibrium constant. In the alkaline range, the equilibrium constant varies with the pH, and its logarithm is a linear function of the charge on the molecule. Since aggregation-disaggregation reactions appear to involve changes at the interfaces of the subunits rather than in the internal structure, the total free energy change in such reactions can be evaluated in terms of the surface free energy change (i.e., calculated from the area and surface charge density). This assumption leads to the correct prediction of the disaggregation constant.

The surface free energy model of oligomer association appears to be useful as a model for the opening and closing of oligomeric channel structures in membranes, and we have used it to describe the voltage gated channels of excitable membranes, in conjunction with a model of the ionic processes in the electrical double layer regions at charged surfaces. The Surface Compartment Model (SCM) of ion flow across the channels of natural membranes emphasizes the role of electrical double layers in ion transport and is derived from first principles. When the SCM is applied to the membrane of an excitable cell, (e.g. the squid axon), one can calculate voltage clamp currents that are similar to those observed in the sodium and potassium channels of excitable membranes. The

difference in the selectivity of the two types of ion channels appears to be determined by the difference in gating current, and is in line with measurements on the sodium and potassium channels of squid axon. These results indicate that there is a kinetic basis for the selectivity of voltage gated channels and suggest that other types of channels may operate by related mechanisms. In summary, our calculations show that: (1) the ionic currents in excitable membranes can be described by electrodiffusion theory, (2) the selectivity of an ionic channel is due to the kinetics of channel opening, and (3) the transient ion concentration changes due to oscillating electric fields can stimulate ion pump enzymes.

3.2 - ELECTROCHEMICAL EFFECTS IN BIOSYNTHETIC STRUCTURES

Recent electrochemical approaches to the mechanisms of oligomeric protein disaggregation and membrane channel opening have been successful in accounting for ion flows during nerve excitation (Blank - BBA 906:277-294, 1987). Since the electrochemical mechanisms are general, they should also operate during the electrical stimulation of transcription and translation in cells. To consider these processes we have adapted the electrochemical model to the interaction of two anionic surfaces with adsorbed counterions in an alternating electric field. The two charged surfaces could represent two DNA's capable of synthesizing mRNA when activated, or an mRNA on a ribosome capable of synthesizing polypeptides. We have examined published data for both RNA and polypeptide synthesis and find that the distributions of molecular weights have the predicted dependence on the frequency of the external electromagnetic field, in support of the proposed mechanism. The electrochemical model predicts other properties (e.g. the properties of new proteins not found in the absence of stimulation) that are being studied further. Because of its generality, it may also provide a rationale for the effects of endogenous electrical stimulation, such as occurs during nerve excitation.

4. LIST OF PUBLICATIONS

4.1 - Blank, M.

Ionic Processes at Membrane Surfaces: The Role of Electrical Double Layers in Electrically Stimulated Ion Transport. in: "Mechanistic Approaches to the Interaction of Electric and Electromagnetic Fields with Living Systems". Edited by M. Blank and E. Findl, Plenum, New York, 1987, pp. 1-13.

4.2 - Blank, M.

Influence of Surface Charge on Oligomeric Reactions as a Basis for Channel Dynamics. in: "Mechanistic Approaches to the Interaction of Electric and Electromagnetic Fields with Living Systems". Edited by M. Blank and E. Findl, Plenum, New York, 1987, pp. 151-160.

4.3 - Blank, M. and Soo, L.

Surface Free Energy as the Potential in Oligomeric Equilibria: Prediction of Hemoglobin Disaggregation Constant. Bioelectrochemistry and Bioenergetics, 17:349-360, 1987.

4.4 - Blank, M. and Goodman, R.

An Electrochemical Model for the Stimulation of Biosynthesis by External Electric Fields. Bioelectrochemistry and Bioenergetics, in press.

5. SCIENTIFIC PERSONNEL

5.1 - Martin Blank, Ph.D.

5.2 - Lily Soo, Ph.D.